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Altered Fatty Acid Metabolism and Reduced Stearoyl-Coenzyme A Desaturase Activity in Asthma

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Abstract

Background: Fatty acids and lipid mediator signaling play an important role in the pathogenesis of asthma, yet this area remains largely under-explored. The aims of this study were (i) to examine fatty acid levels and their metabolism in obese and non-obese asthma patients and (ii) to determine the functional effects of altered fatty acid metabolism in experimental models.

Methods: Medium- and long-chain fatty acid levels were quantified in serum from 161 human volunteers by LC/MS. Changes in stearoyl-coenzyme A desaturase (SCD) expression and activity was evaluated in the ovalbumin (OVA) and house dust mite (HDM) murine models. Primary human bronchial epithelial cells from asthma patients and controls were evaluated for SCD expression and activity.

Results: The serum desaturation index (an indirect measure of SCD) was significantly reduced in non-obese asthma patients and in the OVA murine model. *SCD1* gene expression was significantly reduced within the lungs following OVA or HDM challenge. Inhibition of SCD in mice promoted airway hyperresponsiveness. *SCD1* expression was suppressed in bronchial epithelial cells from asthma patients. IL-4 and IL-13 reduced epithelial cell *SCD1* expression. Inhibition of SCD reduced surfactant protein C expression and suppressed rhinovirus-induced IP-10 secretion, which was associated with increased viral titers.

Conclusions: This is the first study to demonstrate decreased fatty acid desaturase activity in humans with asthma. Experimental models in mice and human epithelial cells suggest that inhibition of desaturase activity leads to airway hyperresponsiveness and reduced anti-viral defense. SCD may represent a new target for therapeutic intervention in asthma patients.

Key words

Fatty acids, asthma, SCD, obesity, epithelial cells

Abbreviations

ACC, acetyl-coA carboxylase; ALI, air liquid interface; ATRA, all trans retinoic acid; BEGM, bronchial epithelial cell growth medium; BMI, body mass index; FAS, fatty acid synthase; HDM, house dust mite; IL, interleukin; IP-10, interferon gamma-induced protein 10; MUFAs, monounsaturated fatty acids; OVA, ovalbumin; PUFAs, polyunsaturated fatty acids; RNA, ribonucleic acid; SCD, stearoyl-coenzyme A desaturase; SFAs, saturated fatty acids; TEER, trans-epithelial resistance.

Introduction

Fatty acid levels and their metabolism can have significant effects on immune cell polarization and inflammatory responses. For example, the metabolism of arachidonic acid by cyclooxygenase enzymes leads to the production of prostaglandins, leukotrienes, and other bioactive eicosanoids, which all have significant effects on the immune system (1-3). Indeed, in obese individuals, an increase in circulating free fatty acids driven by the metabolic overload has been demonstrated to affect immune cells and chronic inflammatory processes (4). Certain fatty acids, e.g. n-3 polyunsaturated fatty acids (PUFAs) or monounsaturated fatty acids (MUFAs), can potentially decrease inflammation through several mechanisms, while saturated fatty acids (SFAs) may contribute to the magnitude of proinflammatory responses (5-7). The balance between pro- and anti-inflammatory fatty acids is increasingly thought to be important in many chronic inflammatory disorders.

It is not only the fatty acid levels themselves that are important, but also the endogenous metabolism of fatty acids, e.g. by elongation and desaturation, will influence their effects in the body. The plasma fatty acid desaturation index (MUFA/SFA ratio) indirectly assesses stearoyl-CoA desaturase (SCD) activity. The SCD enzyme is a rate-limiting enzyme in the synthesis of MUFAs (8) and SCD mutations were identified as the source of the asebia phenotype in mice (9). SCD plays an important role in whole-body energy balance. Notably, skin-specific SCD deletion resulted in increased whole-body energy expenditure, protection against diet-induced adiposity, hepatic steatosis and glucose intolerance, thus linking cutaneous lipid metabolism to whole-body energy balance (10). SCD has also been implicated in the regulation of adipocyte inflammation, macrophage inflammation, myocyte and endothelial cell function (11).

Impaired fatty acid metabolism has been previously proposed to contribute to the pathogenesis of asthma (12-14). One asthma endotype, which frequently presents with more severe clinical features and corticosteroid resistance, is associated with obesity (15-18). Weight loss in obese asthmatics can substantially impact asthma symptoms and airway hyperresponsiveness (19). However, it is likely that metabolic factors, such as free fatty acids, could play a role in the development of asthma in obese and non-obese individuals. In this study we quantified free fatty acid levels in obese and non-obese asthma patients. Circulating levels of many fatty acids were increased in obese patients, but surprisingly we found that the desaturation index was significantly lower in non-obese asthma patients, suggesting reduced SCD activity in non-obese asthma patients. Follow-on studies in animal models and primary human bronchial epithelial cells provide additional functional support for the protective role of this enzyme within the lung.

Methods

Patient groups

This was an observational study in which 161 volunteers were recruited in 4 groups – obese asthma (n=40), obese non-asthma (n=40), non-obese asthma (n=41) and non-obese non-asthma healthy controls (n=40). Asthma patients were recruited only if they had a diagnosis of asthma made by a physician. Severe asthma was defined according to the American Thoracic Society (ATS) Workshop on Refractory Asthma 2000 report and by the 2013 European Respiratory Society (ERS)/ATS guidelines (20, 21). A patient was included to the severe asthma group if he/she fulfilled one or both major and at least two minor ATS 2000 criteria. Patients who did not fulfill these criteria were included in the mild-to-moderate asthma group. Obesity was defined as having a body mass index (BMI) greater than 30

kg/m^2. Non-obese individuals had a BMI of 20-25 kg/m^2. Exclusion criteria were age < 18 or > 65 years, acute infection within 4 weeks of sampling, significant acute or chronic coexisting illness or a condition which contraindicates involvement in the study, malignant or any concomitant end stage organ disease, the use of experimental drugs or the participation in another research study within the past 30 days. Ethical approval was granted at the two recruitment centers (KB-329/2013 and KEK-ZH-Nr. 2012-0443) and all volunteers signed an informed consent. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents. Venous whole blood was drawn from the study participants into tubes without anti-coagulant (BD Biosciences, New Jersey, USA). The tubes were allowed to stand for 2 hours and serum was obtained by centrifuging blood at 800 g for 10 min and was stored at −80 °C until analysis.

Medium- and long-chain fatty acid determination in serum by LC/MS.

Analysis of fatty acids in serum was performed by extraction, derivatization, and analysis by liquid chromatography coupled to mass spectrometry (LC/MS) using the corresponding deuterated fatty acids as internal standards, as previously described with some modifications (22, 23). 20 µL of the mixture of deuterated internal standards at a concentration of 50 µM in EtOH ─ decanoic acid-d_5, undecanoic acid-d_3, dodecanoic acid-d_5, tetradecanoic acid-d_5, pentadecanoic acid-d_3, hexadecanoic acid-d_5, and octadecanoic acid-d_5 (CDN Isotopes, France), as well as palmitoleic acid-d_{14}, oleic acid-d_{17}, α-linolenic acid-d_{14}, arachidonic acid-d_8 and docosahexaenoic-d_5, (Cayman Europe, Estonia) ─ were added to serum samples (250 µL) together with 1 mL of 0.05% H_2SO_4 (v/v aqueous), and 3 mL of ethyl acetate. The mixture was vortexed and centrifuged. The aqueous phase was discarded and the organic layer was evaporated under a stream of nitrogen. The dried samples were

treated with 300 µL of oxalyl chloride (2 M in dichloromethane, Sigma-Aldrich, Spain) and incubated at 65 ºC for 5 min. Samples were evaporated under a nitrogen stream and treated with 250 µL of 3-picolylamine (Sigma-Aldrich, Spain, 1% v/v in acetonitrile). The samples were incubated at room temperature for 5 min, evaporated under a nitrogen stream and reconstituted in 300 µL of EtOH, 80 µL of which were analyzed by LC/MS. LC/MS analysis was performed using an Agilent 1200LC-MSD VL instrument. LC separation was achieved with an Agilent Poroshell 120, SB-C18 column (Agilent, Spain, 2.7 µm, 2.1x50mm) together with a guard column Poroshell 120, UHPLC Guard, SB-C18, (Agilent). The gradient elution mobile phase consisted of A (95:5 water:acetonitrile) and B (95:5 acetonitrile:water) with 0.1% formic acid as the solvent modifier. MS analysis was performed with an electrospray ionization (ESI) source. Fractions were quantified by measuring the area under the peak relative to the corresponding deuterated standard. In all cases, control experiments, with serum enriched with each of the fatty acid under study, were performed to rule out the presence of other metabolites with the same molecular weight or retention time.

Murine models

Female BALB/c mice aged 6-8 weeks were obtained from Charles River (Sulzfeld, Germany) housed and maintained under specific pathogen free conditions at the AO Research Institute, Davos, Switzerland, in individually ventilated cages for the duration of the study. All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals. Experimental procedures were approved by the animal experiment commission of the canton Grisons, Switzerland. Three intraperitoneal immunizations with 20 µg of OVA grade VI (Sigma, Buchs, Switzerland) emulsified in 500 µg Imject™ Alum Adjuvant (Pierce,

Rockford, IL, USA) in 200 µl sterile 0.9 % isotonic Sodium chloride (NaCl) were performed on days 0, 14 and 21, followed by 1 % OVA grade V (Sigma-Aldrich, Buchs, Switzerland) aerosol challenges on days 26, 27 and 28. Mice were euthanized at day 29 for isolation of lung tissue and serum extraction.

In addition, female C57Bl/6 mice (6-8 weeks) were obtained from Charles River and were sensitized intranasally (i.n.) with 1 µg of house dust mite extract (HDM, Greer Laboratories, Lenoir, NC) or NaCl on day 0 and challenged with 10 µg of HDM on days 7-11 before they were analyzed at day 14 for isolation of lung tissue and serum extraction.

Finally, Balb/c mice (from Charles River) were gavaged daily, for 15 days, with 200 µl of NaCl or NaCl containing 0.06mg of the SCD-1 inhibitor MF-438 (Focus Biomolecules, PA, USA). On day 16 mice were intubated under anesthesia and airway resistance was assessed using the FlexiVent system (SCIREQ, Montreal, Canada). Airway resistance was measured in response to increasing concentrations of methacholine (Sigma-Aldrich).

Gene Expression Analysis

RNA from murine lung tissue or bronchial epithelial cell cultures was isolated using RNeasy Mini Kit (Qiagen, Venlo, Netherlands). Reverse transcription of 200 ng RNA was performed with RevertAid RT according to manufacturer instructions (Thermo Fisher Scientific Kit, Waltham, MA USA). Real time PCR was performed with iTaq SYBR Green Supermix (Bio-Rad Laboratories, Hercules, USA) on an Applied Biosystems 7900 HT Fast Real-Time PCR system. The cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles 95°C for 15 sec, 60°C for 1 min, plus an additional step for the dissociation curve. The primers used are listed in Table S1 (in this article's Online Repository) and all

were synthesized by Microsynth (Balgach, Switzerland). The relative expression of each gene was normalized according to the ΔΔCT method.

Bronchial epithelial cell culture

Primary human bronchial epithelial cells were purchased from Lonza (Basel, Switzerland) or were generated from bronchial brushings of asthmatic patients and control subjects, as previously described (24). Cells were cultured as monolayers in Bronchial Epithelial Cell Growth Medium (BEGM; Lonza, Basel, Switzerland) containing Bronchial Epithelial Basal Medium plus all the bullet-kit singlequots at 37°C in a humidified atmosphere at 5% CO_2. Medium was changed every second day. Cells were seeded at a density of 75,000 cells in a 6.5-mm-diameter polyester membrane with a pore size of 0.4 mm (Corning Costar, Corning, NY) in BEGM medium. Once confluent, the medium in the apical compartment was removed and the medium in the basal compartment was substituted with ALI medium (Dulbecco modified Eagle medium [Gibco-BRL, Invitrogen] mixed 1:1 with BEGM supplemented with ATRA 50 nM to allow the cells to differentiate as air liquid interface (ALI) cultures. Trans-epithelial resistance (TEER) was monitored using a Millicell-ERS Volt-Ohm Meter (Millipore, Temecula, CA, USA). SCD1 levels were quantified in cell lysates using an ELISA. The primary anti-SCD1 antibody and secondary HRP conjugated goat anti-mouse antibody were obtained from Abcam (Cambridge, MA, USA).

ALI culture cells were stimulated with the cytokine Th2 cocktail IL-4 and IL-13 (Peprotech, Rocky Hill, USA) at a dose of 10 ng/mL for each cytokine in the basolateral compartment. In addition, ALI cultures were treated with 10 nM of the SCD inhibitor MF-438 (FOCUS Biomolecules), which was also added to the basolateral compartment. After 4 hours incubation with MF-438, cells were apically exposed for 2 hours to rhinovirus at a MOI

of 10 (HRV16; Virapur, San Diego, USA). The apical surface was washed twice with PBS and all remaining liquid removed. Epithelial cell viral titer was quantified using a PCRmax qPCR kit for HRV16, according to manufacturer's instructions. Supernatant IP-10 levels were quantified using a Bio-Plex kit (Bio-Rad, Hercules, USA).

Statistical analysis

Graphing and statistical analysis was performed using Prism 5 (Graph Pad Software, San Diego, CA, USA). Differences between more than two groups were analyzed for statistical significance using one-way ANOVA followed by post hoc analysis. Differences between two groups were analyzed using the Mann–Whitney U test. P-values less than 0.05 were considered statistically significant.

Results

Obesity is associated with elevated circulating levels of fatty acids

Patient demographic and clinical details are included in Table 1. Serum levels of the fatty acids myristic acid (tetradecanoic acid, C14:0), pentadecanoic acid (C15:0) and stearic acid (octadecanoic acid, C18:0) were significantly elevated in obese non-asthma patients compared to non-obese non-asthma controls, while a similar non-statistically significant trend was observed for obese asthma patients compared to non-obese asthma patients (Fig. 1). Palmitic acid (hexadecanoic acid, C16:0), palmitoleic acid (C16:1), α-linolenic acid (C18:3) and docosahexaenoic acid (C22:6) were significantly elevated in both the obese non-asthma and obese asthma patients compared to their respective non-obese controls (Fig. 1). Serum arachidonic acid (C20:4) levels were significantly higher in obese asthma patients compared to non-obese asthma patients, while no differences were observed between obese and non-

obese non-asthma controls (Fig. 1). Serum levels of capric acid (decanoic acid C10:0), undecanoic acid (C11:0) and lauric acid (dodecanoic acid, C12:0) were similar for all groups (Fig. S1 in this article's Online Repository).

We next determined if serum fatty acid levels were associated with any of the patient clinical parameters. Interestingly, serum levels of palmitoleic acid, arachidonic acid and docosahexaenoic acid were significantly reduced in non-obese asthma patients with severe disease (Fig. 2A), which was not observed in obese asthma patients. In addition, the serum desaturation index (palmitoleic:palmitic ratio) was significantly suppressed in non-obese severe asthma patients, with a trend towards reduced levels in mild/moderate non-obese asthma patients, which was not observed in obese asthma patients (Fig. 2B). The serum desaturation index did not correlate with the use or dose of steroids or LABAs (Fig. S2 in this article's Online Repository).

SCD activity is suppressed in murine models of allergic airway inflammation

To better understand the relevance of the suppressed desaturation index observed in non-obese asthma patients, we quantified the desaturation index in mice that were sensitized and challenged with ovalbumin. Similar to the observation in humans, the C16:1 to C16:0 ratio was significantly reduced in the mouse model (Fig. 3A). To confirm this result, the desaturation index using the C18:1 to C18:0 ratio was also calculated. The results were identical (Fig. 3B), suggesting that the metabolism of fatty acids changes following the induction of allergic airway inflammation. The desaturation index reflects the expression and activity of SCD enzymes. Thus the gene expression of the two major murine SCD isoforms, *SCD1* and *SCD2* in the murine lung and liver was quantified. *SCD1* and *SCD2* gene expression were both significantly decreased in the lungs of ovalbumin challenged animals

(Fig. 3C and 3D), while they remained unchanged in the liver (Fig. S3 in this article's Online Repository). In addition, *SCD1* and *SCD2* gene expression were further decreased in the lung following challenge with house dust mite extract (Fig. 3C and 3D). This data suggests that the systemic suppression of the desaturation index, which reflects suppressed SCD activity, may be at least partially due to a allergic inflammation-induced decrease in SCD activity within the lung.

As decreased SCD activity correlates with the presence of airway inflammation, both in humans and mice, the functional consequences for the lung following inhibition of SCD activity was determined. Mice were administered an SCD-1 inhibitor, MF-438, for 15 days and thereafter airway hyper-responsiveness in response to methacholine challenge was assessed. With increasing doses of methacholine, MF-438-treated animals displayed significant defects in tissue elasticity and tissue dampening (Fig. 3E and 3F). The measurement of tissue elastance reflects the energy conservation in the alveoli while the tissue dampening measurement is closely related to tissue resistance and reflects the energy dissipation in the alveoli.

SCD expression is altered in bronchial epithelial cells from asthma patients

SCD gene expression was quantified in primary bronchial epithelial cells from healthy donors (n=4) and asthma patients (n=9). Expression of the two human SCD isoforms, *SCD1* and *SCD5*, was significantly lower in epithelial cells from asthma patients compared to healthy volunteers (Fig. 4A and 4B). In addition to SCD expression, the gene expression of other enzymes involved in fatty acid metabolism was quantified. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, while fatty acid synthase (FAS) catalyzes the synthesis of palmitic (C16:0) from malonyl-CoA. There was no

difference in *ACCalpha* gene expression, but *ACCbeta* was significantly upregulated and *FAS* significantly downregulated in bronchial epithelial cells from asthma patients (Fig. S4 in this article's Online Repository). This data suggests that fatty acid metabolism is significantly disturbed in epithelial cells from asthma patients.

In order to model the cytokine environment of the asthmatic lung, bronchial epithelial cells from healthy donors were incubated with IL-4 and IL-13 for 24 hours. This cytokine cocktail suppressed *SCD1* gene expression and reduced SCD1 protein levels in cell lysates (Fig. 4C). In addition, bronchial epithelial cells from healthy donors were co-incubated with the SCD1 inhibitor, MF-438. SCD1 inhibition did not alter TEER or epithelial cell viability. However, MF-438 significantly reduced surfactant protein C gene expression, with no effect on surfactant protein D expression (Fig. 4D). Lastly, bronchial epithelial cells were infected with rhinovirus (RV16) following treatment with MF-438. Inhibition of SCD1 significantly reduced epithelial cell IP-10 secretion in response to rhinovirus infection, which was associated with an increase in viral titer (Fig. 4E and 4F).

Discussion

Asthma is characterized by recurrent and reversible airflow obstruction with airway inflammation central to its pathogenesis (25). However, successful management of patients with asthma continues to be problematic due to the heterogeneity of the disease with different phenotypes and endotypes continuingly being described (26). In this study, we describe significant differences in circulating fatty acids levels in patients with obese and non-obese asthma. In particular, non-obese patients with severe disease had significantly reduced serum fatty acid levels. In addition, a suppressed MUFA:SFA ratio suggested reduced desaturase activity. Similar to humans, murine models of respiratory allergic inflammation also showed reduced desaturase activity, which correlated with diminished SCD expression in the lung.

Inhibition of SCD had negative effects on lung physiology in the mouse and on primary human bronchial epithelial cell responses *in vitro*.

Our results showing increased serum levels of many different fatty acids in obese individuals is consistent with existing published reports (27). Similarly, serum fatty acid levels were elevated in obese asthma patients compared to non-obese asthma patients. However, the reduction in MUFAs and PUFAs serum levels were only observed in non-obese asthma patients with severe disease and not in obese asthma patients with severe disease. This may suggest a different inflammatory process within the lungs of obese versus non-obese asthma patients, associated with altered metabolism of these fatty acids. Alternatively, dietary differences associated with obesity may compensate for the loss of these fatty acids from the circulation in obese patients with severe asthma. Supplementation of non-obese severe asthma patients with MUFAs or PUFAs may help to restore the availability of these important fatty acids. Clinical studies do show a modest improvement in asthma symptoms following fatty acid supplementation, but truly efficacious treatment schedules are still lacking (28-30).

Further evidence for altered metabolism of fatty acids in non-obese versus obese asthma patients was suggested by the reduced desaturation index in non-obese patients. The SCD enzyme that determines the desaturation index has previously been shown to be important for maintenance of a healthy skin barrier. SCD1 deficient mice exhibit a severe skin phenotype that includes alopecia, atrophy of sebaceous glands, dermatitis and increased permeability of the skin barrier (31). These skin defects were associated with a dramatically altered skin lipid profile and reduced the immunological response to effectively protect against skin-associated bacterial infection (32). Our data now extends the effect of SCD1 blockade to the lung, whereby pharmacological inhibition promoted airway hyperresponsiveness *in vivo* and dampened anti-viral responses *in vitro*. Altered expression

of surfactant protein C in cultured epithelial cells suggested that SCD may support the production of pulmonary surfactant that increases the compliance of the lungs by reducing surface tension. The influence of SCD on the epithelial response to rhinovirus is in line with other reports demonstrating a connection between lipid mediator production and anti-viral responses (33). However, further studies are required to fully address the *in vivo* role of SCD in surfactant production and anti-viral defense within the lung of asthma patients.

Our data suggests that SCD gene expression and the desaturation index may be regulated by inflammatory responses within the lung. We observed decreased lung SCD gene expression in two different murine models of respiratory inflammation (OVA and HDM models) and cytokines such as IL-4 and IL-13 suppress SCD expression in cultured human bronchial epithelial cells. The suppressed desaturation index observed in humans does not seem to be related to the use of corticosteroids as there was no relationship between steroid dose and the index value. In addition, corticosteroids were not used in the murine models. Lastly, we cannot rule out the possibility that SCD gene variants may contribute to a genetic susceptibility to develop more severe airway inflammatory responses. No information is currently available on SCD polymorphisms in asthma. However, a recent genome-wide association study of peripheral blood CD4+ lymphocytes did identify a locus within the Fatty Acid Desaturase gene (*FADS1*/*FADS2*) on 11q, which was associated with asthma susceptibility (34). *FADS1* and *FADS2* encode for Δ5- and Δ6-desaturase enzymes respectively, while SCD is a Δ9-desaturase. Thus, while these enzymes have different targets, this study also implies that inappropriate fatty acid desaturation may play an important role in the development of inflammation within the airways.

In conclusion, fatty acid desaturase activity is reduced in non-obese asthma patients. The functional importance of this change in enzymatic activity will need to be confirmed and further expanded in future studies, but our observations suggest that fatty acid desaturase

activity should be considered an important biological process that contributes to lung inflammatory and immune responses. Significant differences in obese asthmatics compared to non-obese asthmatics continue to be described, further supporting the differential diagnosis and treatment of these asthma endotypes (35-38).

Author contributions

LOM, DM, KS, EH and CA designed the studies and interpreted the data; NS, MK, PS-G, SS and MJ performed the clinical assessments, recruitment of patients and processing of serum samples; NR-P, ES, RFr, RFe, PW, MS, MM-F and OP performed the laboratory procedures. All authors contributed to the preparation and review of the manuscript.

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Conflict of interest

LOM is a consultant to Alimentary Health Ltd and has received research funding from GlaxoSmithKline. CA has received research support from Novartis and Stallergenes and consulted for Actellion, Aventis and Allergopharma. MJ is a consultant to Allergopharma, GER, Anergis, CH, Biomay and received lecture fees from GSK, Allergopharma,

Stallergens, ALK. DM, EH and KS are employees of GlaxoSmithKline and hold company stock. The other authors have no relevant conflicts of interest.

Figure Legends

Figure 1 Serum fatty acid levels are elevated in obese volunteers.

Myristic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, stearic acid, α–linolenic acid, arachidonic acid and docosahexaenoic acid were measured in serum samples from obese and non-obese asthma and non-asthma subjects.

*P < 0.05; **P < 0.01

Figure 2 Disease severity influences serum fatty acid levels

Serum levels of palmitoleic acid, arachidonic acid and docosahexaenoic acid (A) are significantly decreased in non-obese, but not obese, asthma patients with severe disease. In addition, the serum desaturation index (palmitoleic/palmitic acid ratio) was significantly suppressed in the non-obese severe asthma patients (B).

*P < 0.05; **P < 0.01

Figure 3 SCD expression and activity is downregulated in murine models of airway inflammation.

The desaturation index, calculated as palmitoleic/palmitic acid ratio (A) or stearic/oleic acid ratio (B) was suppressed in OVA sensitized and challenged mice (n=8-10 mice per group).

Relative *SCD1* (C) and *SCD2* (D) gene expression was reduced in the lungs of OVA or HDM challenged animals (at least 6 mice and included per group). Methacholine-induced lung elasticity (E) and dampening (F) are increased in mice treated with the SCD inhibitor MF-438 (n=8 mice per group).

*P < 0.05

Figure 4 SCD in human bronchial epithelial cells.

(A) *SCD1* and (B) *SCD5* gene expression are significantly reduced in bronchial epithelial cells from asthma patients (AHBE, n=9 donors) compared to healthy volunteers (NHBE, n=6 donors). (C) IL-4 and IL-13 reduce the levels of SCD1 in NHBE epithelial cells (n=6 donors). (D) The SCD inhibitor MF-438 significantly reduced surfactant protein C (*SftpC*) gene expression, with no effect on surfactant protein D expression (*StfpD*), n=3 NHBE donors. Rhinovirus (RV16) induced less IP-10 secretion (E) but replicated better (F) in MF-438 treated NHBE cells, n=3 donors.

*P < 0.05

Figure S1 Unaltered serum fatty acids.

Figure S2 The serum desaturation index does not correlate with dose of steroids or LABAs used.

Figure S3 *SCD1* gene expression is not altered in liver tissue of murine models of allergic airway inflammation.

Figure S4 Enzymes involved in fatty acid metabolism are dysregulated in bronchial epithelial cells from asthma patients.

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Table 1. Patient Demographics

Obese	No	No	No	Yes	Yes	Yes
Asthma	No	Mild/Moderate	Severe	No	Mild/Moderate	Severe
n=	40	26	15	40	20	20
Age (S.D.)	38.8 (12.1)	39.7 (12.3)	37.7 (9.9)	44.1 (13.8)	47.5 (10.6)	41.9 (12.2)
BMI (S.D.)	22.2 (1.4)	22.8 (1.6)	23.1 (1.1)	36.0 (4.5)	34.9 (4.8)	35.1 (6.0)
Male/Female	15/25	12/14	6/9	15/25	5/15	10/10
Smoker (%)	13 (33)	4 (15)	3 (20)	16 (40)	5 (25)	2 (10)
Alcohol U/wk (S.D.)	2.3 (2.2)	2.5 (3.5)	2.5 (2.9)	1.8 (2.4)	1.4 (2.0)	2.0 (3.1)
FEV1 % (S.D.)	/	80.1 (8.8)	71.1 (9.1)	/	76.6 (7.3)	66.4 (12.3)
Allergy (%)	/	20 (77)	13 (87)	/	15 (75)	16 (80)
Daily ICS (%)	/	21 (81)	15 (100)	/	15 (75)	20 (100)
Daily ICS dose (S.D.)[1]	/	416 (342)	1165 (641)	/	422 (353)	1470 (577)
Oral steroids (%)	/	0 (0)	2 (13)	/	0 (0)	7 (35)
SABA (%)	/	12 (46)	8 (53)	/	12 (60)	11 (55)
LABA (%)	/	14 (54)	12 (80)	/	9 (55)	19 (95)
Daily LABA dose (S.D.)[2]	/	43 (46)	93 (59)	/	45 (49)	128 (81)
Anti-histamines (%)	/	15 (58)	10 (66)	/	10 (50)	15 (75)
Leukotriene receptor antagonist (%)	/	11 (42)	12 (80)	/	9 (45)	15 (75)
Daytime asthma symptoms >2 times(%)?	/	2 (8)	9 (60)	/	2 (10)	13 (65)
Exercise/activity limited due to asthma(%)?	/	5 (19)	12 (80)	/	7 (35)	15 (75)
Waking at night due to asthma symptoms(%)?	/	1 (4)	11 (73)	/	3 (15)	14 (70)
Use of rescue medications >2 times(%)?	/	0 (0)	13 (87)	/	0 (0)	12 (60)

[1]Dose of inhaled glucocorticosteroids calculated as a budesonide equivalent microgram/day

[2]Dose of LABA calculated as a salmeterol equivalent microgram/day

Figure 1

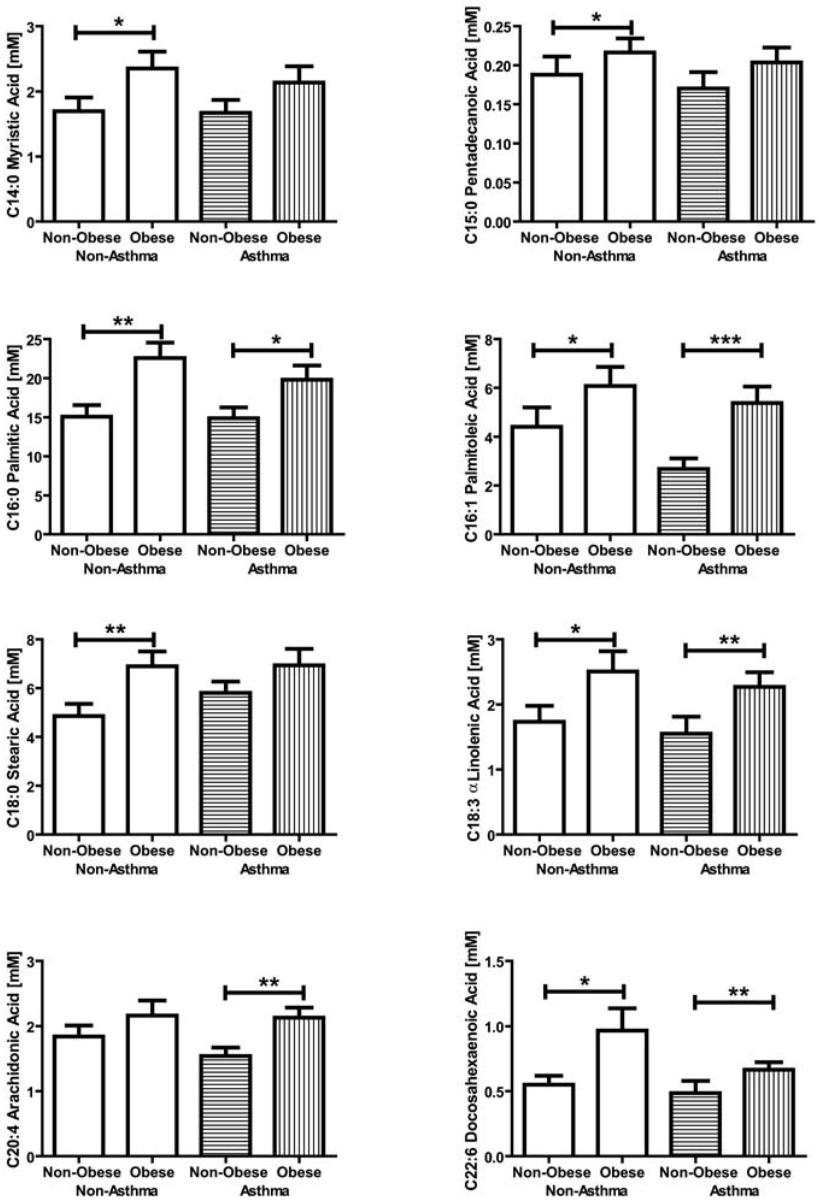

Figure 2

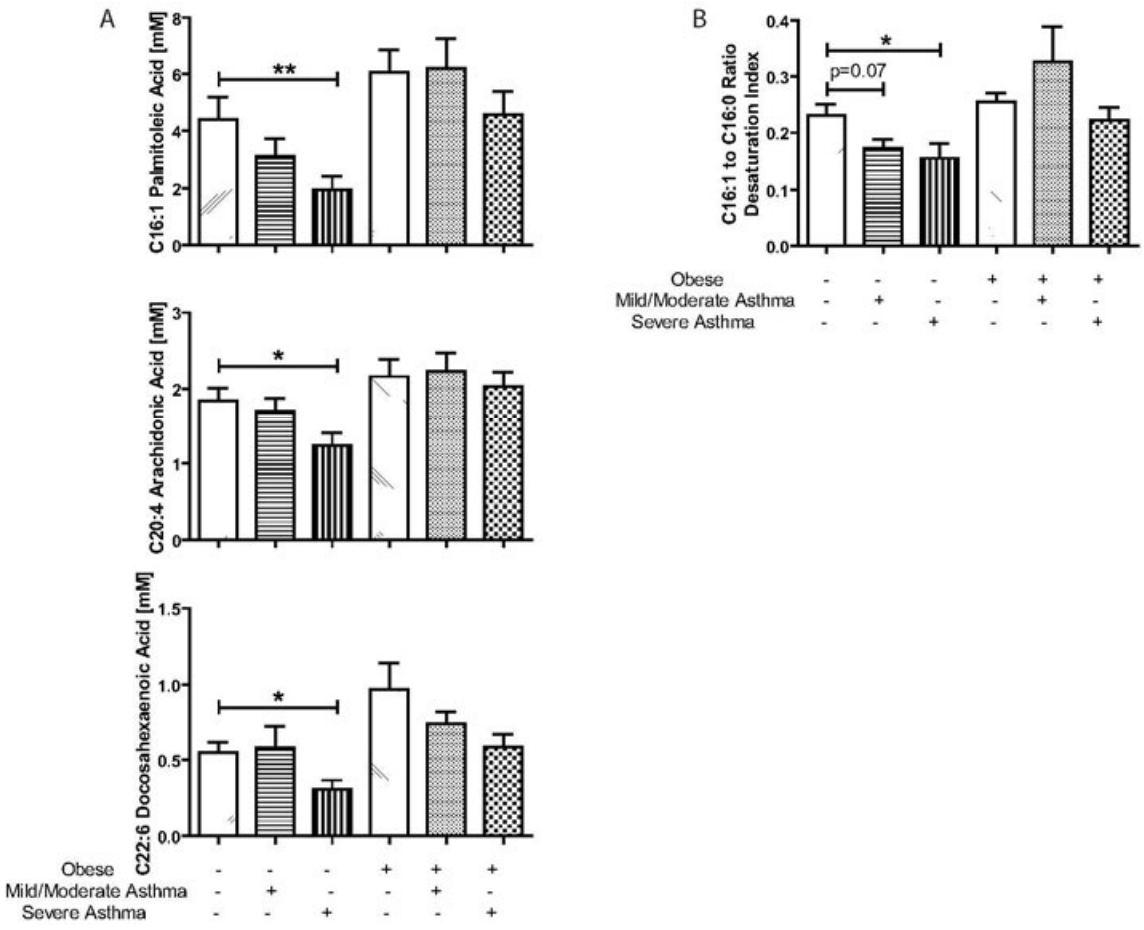

Figure 3

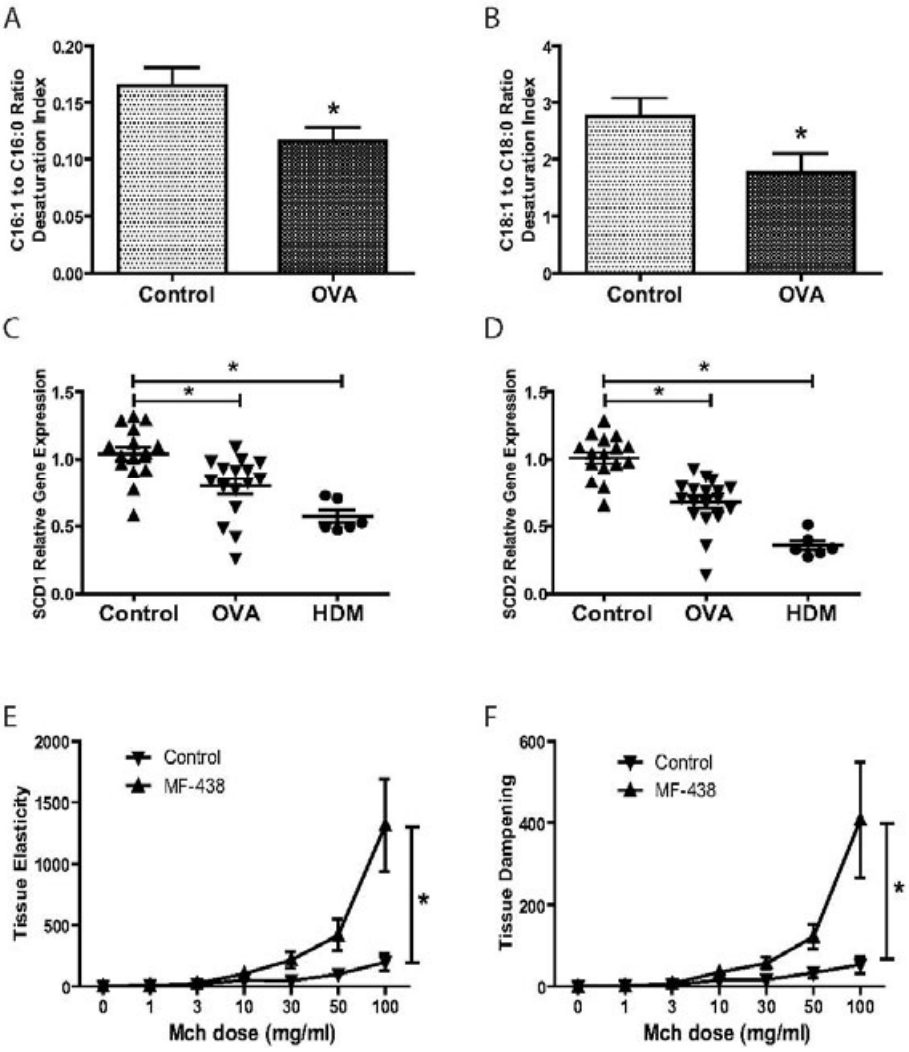

Figure 4